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Clinical Observation

Effects of mild-warming moxibustion on Bcl-2 and PKC expressions of peripheral blood in elderly people

SHI Yin 施茵, CUI Yun-hua 崔云华, WU Huan-gan 吴焕淦, ZHANG Wei 张卫, ZHAO Chen 赵琛, LIU Hui-rong 刘慧荣, GUO Lan-qin 国兰琴, WU Bei-ling 吴蓓玲, YU Ai-zhen 余爱珍, ZHANG Ying-ying 张英英

SHI Yin, CUI Yun-hua, WU Huan-gan, ZHANG Wei, ZHAO Chen, LIU Hui-rong, GUO Lan-qin, WU Bei-ling, YU Ai-zhen, ZHANG Ying-ying, Shanghai University of TCM, Shanghai 201203, China
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Correspondence to: Prof. Wu Huan-gan, Shanghai University of TCM, Shanghai 201203, China. wuhuangan@126.com
Telephone: +86-21-64644238
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Abstract

OBJECTIVE: To explore the anti-aging effects of mild-warming moxibustion on Bcl-2 and PKC expression in peripheral blood and general symptoms in elderly people.

METHODS: A total of 61 elderly people and 30 non-elderly people were enrolled. The total effective rate of mild-warming moxibustion was assessed by symptom scores, and Bcl-2 and PKC expression in peripheral blood was detected by flow cytometry.

RESULTS: The total effective rate in the mild-warming moxibustion group was significantly higher than in the blank control group ($P<0.01$). Bcl-2 and PKC expression rates in peripheral blood in the blank control group were lower than in the normal control group ($P<0.01$), but higher after mild-warming moxibustion ($P<0.01$).

CONCLUSION: The anti-aging effects of mild-warming moxibustion may be due to increased Bcl-2 and PKC expression in peripheral blood in aged people.

Key words: Mild-warming moxibustion; Elderly; Bcl-2; PKC; Clinical study

INTRODUCTION

The aging population has significantly influenced economic growth and medical care. Thus, delaying the aging process has become an important focus. Moxibustion has advantages in delaying aging and has been considered an important method for protecting health. It has proven to play a favorable role in regulating the immune and endocrine systems and in regulating free radicals in the elderly[1-5]. Mild-warming is one kind of moxibustion, indicated for insomnia, chronic visceral hyperalgesia and infertility due to ovulatory disturbance[6-8]. The procedure is to suspend the ignited moxa roll over the acupoint for 10 – 15 min until there is local skin blush, but no pain. In the present study we selected Shenshu (BL 23) and Guanyuan (CV 4) as the effective points[9-10]. Many researchers have confirmed the anti-aging effects of Shenshu (BL 23) and Guanyuan (CV 4), which work by regulating free radicals, the immune system and endocrine function[11-22]. We measured the expression of proteins Bcl-2 (B cell lymphoma gene-2) and PKC (protein kinase C) in peripheral blood of elderly people by flow cytometry so as to provide scientific evidence for using mild-warming moxibustion to prevent and delay the aging process. This study was approved by the appropriate ethics committees and was performed in accordance with the ethical

standards laid down in the Declaration of Helsinki. All persons signed their informed consent prior to their inclusion in the study.

CLINICAL MATERIALS

General Data

A total of 91 subjects, including 61 elderly and 30 non-edlerly participants, were enrolled in the study from September 2006 to April 2007. The elderly were from Shaxiyuan Community of Shanghai, aged 60 – 75 years, who were free of cardiac, cerebral, pulmonary, hepatic, renal and endocrine diseases, and were randomly divided into the mild-warming moxibustion group (n=31) and the blank control group (n=30). In the mild-warming moxibustion group, 10 were male (32.3%) and 21 female (67.7%), aged between 60 and 75 years, with an average age of 68.48±5.32. The average score of aging symptoms was 40.13±3.62, ranging from 33 to 46. In the blank control group, 12 were male (40.0%) and 18 female (60.0%), aged between 60 to 75 years, with an average age of 67.70±4.91. The average score of aging symptoms was 38.80±3.46, ranging from 34 to 45. There were no statistically significant differences in gender, age and the aging symptom score before treatment between the two groups (P> 0.05). The normal control group included 30 non-elderly healthy participants aged 25 – 35 years.

Diagnostic Standards

Elderly people aged from 60 to 75 were selected according to the standards in *Guiding Principles for Clinical Research on Anti-aging Drugs* issued by the Ministry of Health of China[23], which is also based on the classification criteria for elderly people proposed by the World Health Organization (WHO).

TCM Syndrome Differentiation

All elderly subjects had symptoms and signs of kidney-yang deficiency, manifested by flaccidity in loins and knees, aversion to cold, frequent nocturia, lassitude, shortness of breath, loose bowel, loss of hair or graying hair, forgetfulness, loose teeth, decreased sexual desire, edema in the cheeks and eyelids, pale, fat and moist tongue with white-thick coating, and deep-slow pulse with a special weakness in bilateral chi-pulses.

METHODS

Therapeutic Method

The 61 elderly subjects were divided into mild-warming moxibustion group and blank control groups by random number table. The normal control group consisted of 30 normal adolescents. For the mild-warming moxibustion group (n=31), the suspended moxibustion treatment was performed at Shenshu (BL 23) and Guanyuan (CV 4). The two Shenshu (BL 23) acupoints were chosen alternately in combination with Guanyuan (CV 4) point; each treatment lasting 20 min, three times per week, and with 18 sessions making up one treatment course. Changes in clinical symptoms were observed after the first treatment course. Changes in clinical symptoms were observed and the laboratory indexes were measured again after the second treatment course. The blank control group (n=30) received no treatments. Normal control group (n=30) received no treatments.

Observation of Aging Symptoms

Table 1 shows the aging symptoms according to kidney-yang deficiency in the syndrome differentiation for aging found in Appendix 1 of the *Screening Procedures and Standards of Clinical Observations of Anti-aging Chinese Herbs* drawn up by the Third National Conference of the Coordinative Study Group of Integrated Traditional and Western Medicine for the Elderly[24].

Table 1 Syndrome of Kidney-Yang Deficiency

Syndrome	Inspection, Pulse Palpation	Inquiry	
		Main Symptoms	Secondary Symptoms
Kidney-*Yang* Deficiency	1) Edema in Cheeks and Eyelids; 2) Pale, Fat and Moist Tongue proper with White-Thick Coating; 3) Deep-slow Pulse, Weak in Chi-pulse.	1) Flaccidity in Loins and Knees; 2) Extreme Chilliness.	1) Frequent Nocturia; 2) Llassitude; 3) Shortness of Breath; 4) Loose Stool; 5) Graying Hair or Hair Loss; 6) Forgetfulness; 7) Loose Teeth; 8) Decreased Sexual Desire.

Table 2 The therapeutic effects

Groups	n	Marked Effect	Improvement	Failure	Effective Rate (%)
Mild-warming Moxibustion Group	31	1	26	4	87.10**
Blank Control Group	30	0	3	27	10.00

Note: As compared with the blank control group. ** P<0.01.

Scoring Standards of the Clinical Symptoms

Assessment of clinical symptoms was based on the assessment method for scoring aging symptoms in the ap-

pendix of the *Screening Procedures and Standards of Clinical Observations of Anti-aging Chinese Herbs* drawn up by the Third National Conference of the Coordinative Study Group of Integrated Traditional and Western Medicine for the Elderly[24], as well as according to the criteria of scoring aging symptoms in the clinical assessment for anti-aging drugs of the *Modern Pharmaceutics for the Elderly*[25]. The final aging score was the cumulative score for each of the single symptoms.

Inquiry: All participants were asked the same questions. A score of 4 would be given if the symptom was volunteered; the score was 3 if the symptom was obtained by inquiry and the symptom occurred significantly or continuously; symptoms which were paroxysmal or unstable were scored 2; if the symptoms were mild or appeared occasionally, the score would be 1; the score would be 0 if there were no symptoms.

Inspection and pulse palpation: A score of 6 was given if each of the symptoms existed.

For assessment of the aging symptoms of kidney-*yang* deficiency, the patient had to have two of the main symptoms, 3 of the secondary symptoms, and 1 symptom each, in the inspection and pulse palpation, with a total score ≥ 13.

Standard for Therapeutic Effects

This was according to the therapeutic effect criteria in appendix 2 of the *Screening Procedures and Standards of Clinical Observations of Anti-aging Chinese Herbs* drawn up by the Third National Conference of the Coordinative Study Group of Integrated Traditional and Western Medicine for the Elderly[24]. Marked effect: the aging score after treatment was reduced by > 2/3. Improvement: the post-treatment aging score was decreased by 1/3 – 2/3. Failure: the post-treatment aging score was reduced by <1/3.

Detection of Bcl-2 and PKC Expression

At the end of the second treatment course, a fasting peripheral blood sample was collected and the expression of Bcl-2 and PKC was detected by flow cytometry.

According to protocol, 10 μL CD45-PC5 was added to 50 μL heparin-anticoagulated peripheral blood and incubated for 15 min at room temperature, then agitated and mixed with 100 μL of IntraPrep Reagent 1 and incubated for another 15 min at room temperature away from light. Afterwards, 4 mL of PBS-BSA was added and the sample centrifuged at 300 g/min for 5 min, discarding the supernatant. Next, 100 μL of IntraPrep Reagent 2 was added, mixed gently and incubated at room temperature for 5 min away from light. The primary antibody was added by corresponding dosage, gently mixed until homogeneous, and incubated for 20 min at room temperature away from light. Four mL of PBS-BSA was added and the supernatant was discarded following centrifugation for 5 min at 300 g/min. One mL of PBS-BSA was added and resuspended before examination if direct fluorescent staining was positive. If indirect fluorescent staining occurred, 2 μL of secondary IgG-FITC antibody was added, gently mixed until homogeneous, and incubated at room temperature for 20 min away from light. Four mL of PBS-BSA was added and the supernatant was discarded after centrifugation for 5 min at 300 g/min. One mL of PBS-BSA was added and the sample resuspended, then examined for Bcl-2 and PKC expression.

For the control group, CD45-PC5 was added but with no antibodies. For the blank group, CD45-PC5 and the second antibody or IgG1 (mouse) – FITC was added.

Statistical Method

SPSS11.0 software was used for statistical processing. The measurement data were assessed using *t*-tests and the categorical data were assessed by Chi-square tests. The percentage of cells in each stage of the cell cycle was expressed as ($\bar{x}$ ±s)%, and assessed using one-way ANOVA. The Kruskal-Wallis test was used if values were not in accord with the conditions of ANOVA.

RESULTS

Therapeutic Effects

As shown in Table 2, in the 31 participants in the mild-warming moxibustion group, 1 obtained a marked effect, 26 improvement, and 4 showed failure; with a total effective rate of 87.10%. Of the 30 participants in the blank control group, no participants obtained a marked effect; 3 improvement; 27 no improvement; with a total effective rate of 10.00%. There was a statistically significant difference between the two groups (P<0.01).

The Effect of Mild-warming Moxibustion on Bcl-2 Expression in Peripheral Blood in the Aged

As shown in Table 3, the Bcl-2 expression rate in peripheral blood in the aged was significantly lower than in the normal control group (P<0.01). There was a significantly higher Bcl-2 expression rate in the mild-warming moxibustion group than in the blank control group (P<0.01).

The Effect of Mild-warming Moxibustion on PKC Expression in Peripheral Blood in the Aged

As shown in Table 4, the PKC expression rate in peripheral blood in the aged was significantly lower than in the normal control group (P<0.01). There was a significantly higher PKC expression rate in the

mild-warming moxibustion group than in the blank control group ($P<0.01$).

Table 3 The Bcl-2 expression rate in peripheral blood in each of the groups ($\bar{x}$ ±S)%.

Groups	N	Positive expression of protein Bcl-2 (%)
Blank control group	30	33.45 ± 4.04 **
Mild-warming moxibustion group	31	41.75 ± 3.23 $^{**\Delta\Delta}$
Normal control group	30	59.95 ± 7.14

Note: Compared with the normal control group, $^{**}P<0.01$; compared with the blank control group, $^{\Delta\Delta}P<0.01$.

Table 4 The PKC expression rate in peripheral blood in each of the groups ($\bar{x}$ ±S)%.

Groups	N	Positive expression of protein PKC (%)
Blank control group	30	3.29±2.29**
Mild-warming moxibustion group	31	22.78±6.30$^{**\Delta\Delta}$
Normal control group	30	38.68±6.42

Note: Compared with the normal control group, $^{**}P<0.01$; compared with the blank control group, $^{\Delta\Delta}P<0.01$.

DISCUSSION

Telomerase, an RNA-directed DNA polymerase that may extend telomeres of eukaryotic chromosomes, plays an important role in aging and anti-aging[26-36], which is a new important highlight in the genetic program of aging. Several proto-oncogenes and tumor suppressor genes are implicated in the regulation of telomerase activity, including Bcl-2 and PKC[37-40]. B cell lymphoma gene-2 (Bcl-2) is an important anti-apoptotic gene, residing at human chromosome 18q21, and containing three exons, 230 kb in length. Gene Bcl-2 encodes a protein of 25 – 26 KD Previous studies revealed that Bcl-2 usually resides in the mitochondria, endoplasmic reticulum, and nuclear membrane where a high number of oxygen free radicals are produced, related to anti-oxidation and self-protection of the cells. Bcl-2 is also call "the longevity gene", for its resistance to apoptosis induced by various stimuli, and is a common index adopted to detect aging[41]. The expression of Bcl-2 is decreased in aged cells[42-51]. Some experiments have demonstrated that Bcl-2 may prolong the life of many types of hematopoietic growth factors[52].

Protein kinase C (PKC) is a type of Ca^{2+} phospholipid-dependent protein kinase, involved in the modulation of cell actions[53]. Activated PKC can phosphorylate Ser or Thr residues in protein substrates, and is one of the important molecules in the signal pathway. A number of experiments have implied that dysfunction of PKC plays a decisive role in the development and process of senescence in aged cells[54-64]. PKC is widely distributed in the organs, tissues and cells of mammals, influencing the transmission of intracellular biological signals, and plays an important role in the modulation of cellular metabolism, differentiation, proliferation and even apoptosis[65-69]. PKC is considered one of the key molecules in intracellular signal transmission, because it can activate target enzymes in the cytoplasm to take part in the regulating of biochemical reactions and it can also act on transcription factors in the cell nucleus to modulate the expression of genes related to growth and differentiation of cells.

Previous research shows that high expression of Bcl-2 and PKC may raise telomerase activity, and PKC may promote Bcl – 2 expression. Telomerase activity can be adjusted by PKC through effecting hTERT expression[70]. Mitochondrial apoptosis induced by hTERT is related to the inhibition of Bcl – 2 expression[71-72]. Bcl-2 and PKC may adjust telomerase activity alone or synergistically.

In the present study, the anti-aging effects of mild-warming moxibustion were observed by scoring the general symptoms and detecting Bcl-2 and PKC expression in peripheral blood in the aged. The results showed that the total effective rate was higher in the mild-warming moxibustion group than in the blank control group ($P<0.01$); Bcl-2 and PKC expression rates in peripheral blood were lower in the blank control group than in the normal control group; while they were higher in the mild-warming moxibustion group than in the blank control group. Mild-warming moxibustion may delay the aging process by increasing Bcl-2 and PKC expression in peripheral blood in the aged. Therefore, mild-warming moxibustion is worth popularization in clinics. However, the relationship of the relevant indexes with the anti-aging effects of mild-warming moxibustion and the molecular mechanisms involved needs further study.

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